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Intra-annual variation in biochemical properties of different grassland soils under contrasting management and climate

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Abstract

Soil biochemical properties are important bio-indicators of soil quality. In the present study, the effects of different management practices on soil biochemical properties were evaluated in three different pairs of grassland soils (unmanaged and managed) located in three areas of Galicia (NW Spain) where different types of climate prevail. Variations in soil biochemical properties were monitored throughout one year. Changes throughout the year in soil temperature, soil moisture content and particularly soil location and soil management affected the values of the biochemical properties. Comparatively higher levels of enzyme activity were observed in the unmanaged grasslands than in the managed grasslands, especially for β-glucosidase and the enzymes involved in the P and S cycles (phosphodiesterase, phosphomonoesterase and arylsulphatase). A biochemical equilibrium index was used to evaluate soil quality. Although variations in the index were observed throughout the year, the values mainly depended on soil management

and revealed that unmanaged grasslands were in a situation of biochemical equilibrium throughout the study period, while no such equilibrium was observed in managed grasslands for most of the study period.

Keywords: Soil quality; soil enzymes; soil biochemical properties; annual variation; grassland soils; soil management.

Introduction

Soil biological properties are used as indicators of soil quality because they are sensitive to any alterations that the soil may undergo (Yakovchenko et al., 1996) and because the biological components of soil are essential to many soil processes and functions (Dick et al., 1996). A further advantage of the use of soil biological properties rather than physical and chemical properties as indicators of soil quality is that the former are relatively rapid to measure and are very sensitive to environmental changes.

As a result of the interest in determining how biochemical properties can be used to assess soil quality, many studies have addressed the effects of management practices on soil biochemical properties, and these are now generally well established. For example, Lovell and Hatch (1998) and Ge et al. (2009) considered the effects of fertilisers, Tracy and Frank (1998) considered different grazing systems, and Ekenler and Tabatabai (2004) differences in tillage treatments. Despite the advances made in this field, there are still some major drawbacks associated with the use of soil biochemical properties as indicators of soil quality. Firstly, information about how soil biochemical properties respond to combinations of management practices in grassland soils is scarce; secondly, although there is some information about the effects of management, the intra-annual variation in soil biochemical properties has not been well studied. Finally, another disadvantage is the high spatial and temporal variability often observed in soil

biochemical properties, which contrasts with the effects of management on the same properties and hinders their use as indicators of soil quality.

Extracellular enzymes can be stabilized by clays and humus in soil, and are involved in decomposition processes that are affected by edaphic factors (Burns, 1982). Because of the diverse origins, locations and functions of different enzymes, their activities are expected to display wide temporal variations of diverse magnitude and different patterns of natural variability. Some microorganisms are more resistant to disturbance and thus will tend to fluctuate less in response to changes in environmental conditions (Tilman, 1996). Thus, the temporal variability in soil biochemical properties is, to a certain extent, a measure of the resilience of the soil microbial community (Wardle, 1998). On the other hand, soil is a dynamic, living system in which biological communities are constantly changing, mainly due to variations in temperature, soil moisture, pH and nutrient supply, which will cause biochemical properties to change. Although climate parameters have been regarded as one of the main factors that affect soil biological and biochemical properties (Insam, 1990; Wardle, 1998), the results of some studies that have examined the seasonality of soil biochemical properties have been contradictory. For example, Holmes and Zac (1994) and Ross et al. (1995) reported no differences in the size of the microbial biomass in relation to season, while Monokrousos et al. (2004) reported significant seasonal variations in many soil microbial properties. Finally, it is important to analyze a wide range of properties in seasonal studies of soil biochemical properties, as the results of single assays may not be representative of the overall activity of the microbial community.

Within the above theoretical and experimental considerations the overall aim of the present study was to examine the effects of season and soil management on soil

biochemical properties in temperate grasslands. The study specifically addressed the following concerns: (a) the abiotic factors that drive the temporal variations; (b) the importance of temporal variations in temperate grassland soils; (c) the effects of the combination of management practices (managed compared with unmanaged grassland) on the values of soil biochemical properties, (d) development of an expression that will enable quantification of soil quality in grassland ecosystems, but which at the same time is independent of seasonal variations.

Material and methods

Soils

Six small experimental plots (of size between 0.5 and 1 ha) located in Galicia (NW Spain) were used in the study. The plots were located at three different sites with different climatic conditions, and a fertilised (managed grassland) and an unfertilised plot (unmanaged grassland) were selected for study at each site. All of the grassland soils under study are classified as Umbrisols (ISSS Working Group R.B., 1998). Two of the grasslands were located at Boimorto (8º 7´ 28´´ W and 43º 2´ 3´´ N) at an altitude of 445 m.a.s.l. Another pair of grasslands was located at Trabada (7º 10´ 42´´ W, 43º 24´ 38´´ N), at 240 m.a.s.l. The other site was located at Rodeiro (7º 58´ 17´´ W 42º 41´ 27´´ N), at an altitude of 620 m.a.s.l. Some general properties (parent material, pH, organic matter content and texture) of the soils are shown in Table 1.

The daily average temperature and daily rainfall were obtained from three meteorological stations located near the study plots. The climatograms obtained for the three locations during the period of study are shown in Fig. 1.

The vegetation in the unmanaged grasslands was dominated by plant species characteristic of poorly fertile soils, including *Agrostis capillaris* L., *Holcus lanatus* L.,

Anthoxanthum odoratum L., *Lolium perenne* L. and *Poa annua* L., with a scarce presence of legumes, mainly *Trifolium repens* L. The vegetation in the managed grasslands was dominated by *Lolium perenne* L. and *Trifolium repens* L. The unmanaged grassland had never been tilled, whereas the managed grassland had been tilled when grass was seeded (every 2-4 years). Both, managed and unmanaged plots, were grazed by cattle. The unmanaged grasslands had never been fertilised, and the managed grasslands were fertilised as follows: in the Boimorto plots, organic slurry (containing 25 kg ha^{-1} N and 8 kg P ha^{-1}) was applied in January and May 2004 and inorganic fertiliser (80 kg N ha^{-1} and 20 kg P ha^{-1}) was applied in September 2004. In the Trabada plot, cattle slurry was applied in December 2003 and June 2004 (equivalent to the addition of 60 and 80 kg N ha^{-1} and 18 and 24 kg P ha^{-1}, respectively). Finally in the Rodeiro plots, inorganic fertiliser (170 kg N ha^{-1} and 30 kg P ha^{-1}) was applied in April 2004.

Soils were sampled monthly in the last week of October, November and December in 2003, and in the last week of January, February, March, April, May, June, July, August, September and October in 2004. In each of the six plots, a representative sample was obtained with a shovel, from the top 10 cm of the upper horizon at 10-15 points distributed uniformly over the whole area of the plot. Approximately five kilograms of soil were sampled in each plot. Samples were pooled in the field to obtain composite samples representative of each site, which were transported in isothermal bags to the laboratory and sieved (< 4 mm). The moisture content was determined gravimetrically. A sub-sample of soil was air-dried to ascertain the general soil properties, and the remaining soil was stored at 4 ºC pending biochemical analyses. All biochemical analyses were carried out within two weeks of sampling.

Analytical methods

Total C (dichromate oxidation) and N (Kjeldahl digestion) contents, pH in water (1:2.5 soil:water ratio) and in 0.1 M KCl (same ratio as for pH in water), and particle size distribution and texture were determined following the methods described by Guitián and Carballas (1976).

Soil microbial biomass C (biomass-C) was determined by the chloroform fumigation-extraction method (Vance et al., 1987). The difference in the C content of the fumigated and unfumigated extracts was converted to microbial biomass C (expressed in mg kg^{-1} of dry soil) by applying a factor (K_c) of 0.45. The C extracted with K_2SO_4 from the unfumigated samples was used as a measure of the labile pool of C.

Soil basal respiration (mg CO_2–C kg^{-1} h^{-1}) was determined by static incubation (Guitián and Carballas, 1976). The CO_2 produced during a 10-day period by 25 g soil samples incubated at field moisture content and at 25 ºC was collected in 10 ml of a 1 M NaOH solution, which was then titrated against HCl, with an automatic titrator.

To determine net N mineralisation, soil samples (10 g) were extracted for 30 min with 50 ml of 2 M KCl before and after incubation for 10 days at 25 ºC at field moisture content. Total inorganic N was determined in the extracts by Kjeldahl distillation (Bremner, 1965). Net nitrogen mineralisation (mg kg^{-1} 10 d^{-1}) was calculated as the difference between the values obtained before and after incubation.

Dehydrogenase activity was determined as described by Camiña et al. (1998), and the results were expressed as µmol iodonitrotetrazolium formazan (INTF) g^{-1} h^{-1}. Catalase activity was determined according to the method of Trasar-Cepeda et al. (1999) and the results were expressed as mmol H_2O_2 consumed g^{-1} h^{-1}.

Acid phosphomonoesterase, β-glucosidase, phosphodiesterase and arylsulphatase activities were determined following modifications of the original methods, as described by Trasar-Cepeda et al. (2008). The activity of each of these four enzymes was expressed as µmol *p*-nitrophenol g^{-1} h^{-1}.

The activities of urease and protease hydrolysing benzoylargininamide (BAA-protease) were determined as described by Nannipieri et al. (1980). In both cases, enzyme activity was expressed as µmol NH_3 g^{-1} h^{-1}.

The activity of protease hydrolysing casein (casein-protease) was determined as described by Ladd and Butler (1972). Enzyme activity was expressed as µmol tyrosine g^{-1} h^{-1}.

Invertase and carboxymethylcellulase (cellulase) activities were determined following the method of Schinner and von Mersi (1990). Both activities were expressed as µmol glucose g^{-1} h^{-1}. All enzyme activities were determined in triplicate.

Soil quality assessment

The following equation, developed by Paz-Ferreiro et al. (2007) for native grassland soils in Galicia, was used to assess biochemical equilibrium:

Total carbon = 0.764 + (2.304 10^{-3} biomass-C) + (0.936 catalase activity) + (0.017 urease activity) + (0.206 phosphomonoesterase activity) Equation 1

where: total C is expressed as a percentage, microbial biomass C in mg kg^{-1}, urease and phosphomonoesterase activities as µmol of product released g^{-1} h^{-1} and catalase activity as mmol H_2O_2 consumed g^{-1} h^{-1}.

Soil biochemical equilibrium was estimated by comparing the total carbon content measured by the dichromate oxidation method (Cr), with the total carbon content estimated from the equation (Ct). Theoretically, the value of the 100 Ct/Cr ratio in soils

in biochemical equilibrium should be 100 (this means the same carbon content determined in the laboratory from the dichromate oxidation method and estimated from soil biochemical properties using equation 1) although it has been shown that in natural undisturbed grasslands in Galicia, the values range from 85 to 115 (Paz-Ferreiro et al. 2007). Thus, values of the 100 Ct/Cr ratio of between 85 and 115 are considered typical of high quality grassland soils.

Statistical analysis

All statistical analyses were performed with SPSS, version 15.0. Differences in mean values were tested by analysis of variance (ANOVA), with management and location as variables, and average daily temperature at the study site during the week prior to the sampling and soil humidity at the moment of sampling as covariables.

A Principal Component Analysis (PCA) was carried out for all the biochemical variables measured and for soil organic matter content. Factors were extracted by varimax rotation. An ANOVA was also carried out to test differences between treatments in the factor analysis (using factor 1 of the PCA, which explains the highest variability).

Discriminant analysis (DA) was used to assess how well samples taken under different location and management can be separated on the basis of all of the properties analyzed. This method was used to evaluate the impact of individual variables distinguishing different samples. To test for significance differences in distances between group centroids, the P-levels associated with Mahalanobis distances were estimated.

Results

Organic matter, pH and biochemical properties

At all three experimental sites, organic matter contents were significantly lower in the managed grasslands (see Table 1) than in the unmanaged grasslands (P<0.001 for both organic carbon and total nitrogen). They also varied depending on the location of the soil analyzed (P<0.001) in the order Rodeiro > Boimorto > Trabada. Soil pH was not affected by grassland management or location (see Table 1) and varied in the order Rodeiro > Trabada > Boimorto for the managed grasslands and in the order Boimorto > Rodeiro > Trabada for the unmanaged grasslands.

In general, the values of the biochemical properties varied widely. The activities of the soil biochemical properties were generally lower in managed plots than in unmanaged plots (Fig. 2, 3, 4).

The results of the ANOVA revealed that all 15 of the soil biochemical properties measured, with the exception of cellulose activity, were affected by location (Table 2). All soil biochemical properties, with the exception of net mineralised N were affected by management. Finally, the interactive effect between management and location was significant in 9 out of the 15 variables (C-biomass, labile C, dehydrogenase, cellulase, β-glucosidase, invertase, casein-protease, phosphodiesterase and arylsulphatase).

The ordination of the samples and variables on a PCA plot is shown in Fig. 5. The first two axes of the PCA plot accounted for 68% of the data variability (60% of the variability was explained by the first axis, while only 8% of the variability was explained by the second axis). Samples from unmanaged grasslands appeared on the right side of the biplot, while samples from the managed grasslands appeared on the left side of the plot. The factor analysis placed the managed grassland of Boimorto and Trabada together and significantly separated from all the other samples. The same applied to the managed grassland at Rodeiro and the unmanaged grassland at Boimorto.

The unmanaged grasslands at Trabada and Rodeiro appeared on the positive side of Factor 1 and significantly separated from the other groups (and also significantly separated from each other) (Fig. 5). All the hydrolases (excluding cellulase) and oxidoreductases, C-biomass, basal respiration, labile carbon and organic carbon had the greatest weighting in Factor 1. An ANOVA (not shown) demonstrates that Factor 1 was significantly influenced by temperature ($P<0.01$) and by location and management (in both cases $P<0.001$) (Fig. 5), while Factor 2 was significantly influenced by location and management ($P<0.001$). Ordination of the samples in the PCA biplot showed that most of the variability in the samples can be attributed to soil management, location of sample and seasonal variability. The ordination of samples from Trabada and Boimorto was more similar to that of samples from Rodeiro.

Discriminant Analysis revealed that five discriminatory functions contributed significantly to separation of the soil samples, although two of them accounted for 94% of the total variation (Fig. 6). All 78 samples considered in the study were correctly classified, except one of the samples of the unmanaged soil from Boimorto, which was classified as a managed sample from Trabada. Moreover, all distances between group centroids were significantly different from each other ($P<0.05$). In the first root, the highest absolute values of standardized coefficients obtained were for phosphomonoesterase, arylsulphatase, invertase and β-glucosidase (Table 3), while in the second root the corresponding highest values were those obtained for arylsulphatase and phosphodiesterase, followed by invertase, labile C, net mineralised N and cellulase.

The variability in the 100 Ct/Cr ratio for the six soils analyzed and for the different sampling dates is shown in Fig. 7. In this case, 100 Ct/Cr value in the unmanaged grassland was in the range of 82 to 115 (typical for unmanaged, native grasslands: Paz-

Ferreiro et al., 2007), while the range was between 42 and 178 in the managed grasslands. In the case of managed grasslands there was a difference in the values obtained for Trabada, which ranged from 79 to 178 and, on the other hand, the values obtained for Boimorto (42-88) and Rodeiro (63-105). The differences in the values of the 100 Ct/Cr ratio in the three managed grasslands were significant (P<0.05) and there were also significant differences associated with the location of soil samples (Table 4). Temperature and humidity did not have a significant effect on the value of the Ct/Cr ratio.

Discussion

Annual variation in soil biochemical properties and the influence of abiotic factors

The activities of the biochemical properties under study varied between sampling dates and with soil location (Figs. 2, 3 and 4). The monthly variation in soil biochemical properties was very high, and in some isolated cases the values of the soil biochemical properties doubled or halved from one month to the next. The lower values of biochemical properties in managed grasslands are consistent with results of previous studies (Zeller et al., 2001; Paz-Ferreiro et al., 2009).

The average coefficients of variation differed among the different properties measured throughout the year, and generally ranged from 20 to 30% for most of the properties analyzed, although the coefficients were higher than 30% for urease and net N mineralisation and lower for labile carbon and phosphomonoesterase. The low values of the coefficient of variation for phosphomonoestase activity are consistent with those reported by Dick et al. (1988) and Bolton et al. (1985) for the same enzymatic activity, although the latter study also reported very low annual variations in urease activity (< 10%). By contrast, other authors reported coefficients of variation of around 50% for

many biochemical properties (Debosz et al., 1999; Waldrop and Firestone, 2006). In a review of the seasonality of soil microbial biomass, Wardle (1998) reported coefficients of variation of between 4 and 91%. This indicates that the coefficient of variation of a soil biochemical property greatly depends on the property considered, but also on other factors such as type of climate, vegetation and substrate.

No seasonal pattern of variation in the activities of the biochemical properties under study was observed in any of the six soils studied. Other authors have also reported no clear annual patterns of variation for many soil biochemical properties (Dick et al., 1988). However, some of the biochemical properties studied depended on parameters related to climate (soil moisture on the sampling date and mean average temperature during the week prior to sampling). Thus only dehydrogenase and invertase activities were significantly and positively affected by soil humidity at sampling time (Table 2). With respect to the scarce effect of soil humidity on biochemical properties, this was not surprising, as authors such as Bandick and Dick (1999) have reported that some enzymatic activities scarcely varied in relation to soil moisture in similar ecosystems. On the other hand, it has also been shown that drought can inhibit some enzyme activities (Sardans et al., 2008). Although the temperature on the day of soil sampling was not a significant factor in explaining the values of soil biochemical properties (data not shown), the effect of the mean average daily temperature during the week prior to sampling was a significant factor explaining the variation in the values of biomass-C, labile C, basal respiration, net N mineralisation and in the activities of P and S cycle enzymes (phosphomonoesterase, phosphodiesterase and arylsulphatase) (Table 2). The activity of enzymes involved in the P and S biogeochemical cycles has previously been reported to be strongly related to temperature (Li and Sarah, 2003; Paz-Ferreiro et al.,

2010) and interestingly, the enzymes involved in the carbon and nitrogen cycles were not dependent on temperature. Differences in the values of the biochemical properties at the three locations sampled may be explained by the existence of a temperature gradient at the sites, in the order Rodeiro < Trabada < Boimorto. Temperature can influence enzyme activity by direct modification of enzyme kinetics (Trasar-Cepeda et al., 2007) as well as by indirect effects on microbial proliferation, and may explain some of the variability in the biochemical properties (particularly in the P and S cycles).

Influence of location and management on soil biochemical properties

Several long term and intermediate term studies have shown that soil biochemical properties can distinguish the effects of soil management practices (Bandick and Dick 1999; Paz-Ferreiro et al., 2009). However, according to van Diepeningen et al. (2006), soil characteristics are much more important in determining microbial structure and function, and therefore have a greater influence than management on the values of soil biochemical properties. In the present study, management was found to have an important effect on soil biochemical properties (Table 2), and location a less important effect, possibly due to differences in soil characteristics among sampling sites.

The organic matter content and clay content of the Rodeiro soils is higher than that of the Trabada and Boimorto soils. The higher values of these two soil properties may have resulted in higher values of biochemical properties in the Rodeiro soils. The influence of location on soil biochemical properties is probably due to diverse soil formation factors. Thus parent material, vegetation and relief caused differences in texture, soil organic matter content, substrate availability, and perhaps microbial community structure. On the other hand, climate also affects soil microbial activity, as clearly observed in the present results (see previous section).

With respect to management, prior to the study the managed soils had been tilled biannually. Tillage is known to favour the breakdown of soil organic matter through increased aeration and the breakdown of soil aggregates, which implies the exposure of previously inaccessible organic matter to microbial attack. This results in lower levels of soil organic matter and lower biochemical activity in tilled soils. As previously reported (Haynes, 1999), the present results suggest that tillage and grassland management practices have similar overall effects on the activities of the different enzymes involved in the cycling of C, N, P and S in soils, as observed by the strong relationship between Factor 1 identified in the PCA analysis and soil management (ANOVA, significant at $P<0.001$, data not shown).

The effects of management and soil location were revealed by the Discriminant Analysis, which was able to separate the six soils correctly, on the basis of the values of their biochemical properties.

Modification of soil quality by grassland management

The biochemical soil quality index indicates that managed grasslands at Boimorto and Rodeiro are biochemically degraded (100 Ct/Cr < 85%), while the managed soil at Trabada oscillates from values indicating biochemical enrichment (100 Ct/Cr >115%) to values indicating biochemical degradation. The high Ct/Cr ratio in the managed field at Trabada may indicate a transient state of high microbiological and biochemical activity due to the abundant use of organic amendments, and can be attributed to recent use of organic amendments, as the peaks in activity were produced shortly after the addition of slurry. The quality of the managed grassland soil at Rodeiro appears to be the highest of the managed grasslands considered in this study, as the variations in the

100 Ct/Cr index are much lower than in the other two managed plots and the mean value (106±8) is closer to the equilibrium value of 100.

The index was not significantly affected by either temperature or soil humidity so that it can be considered quite robust and, to a certain extent, independent of seasonal changes (Table 4). However, management and the interaction between management and location did have significant effects on the index, showing that it is sensitive to management and that the different management practices carried out in the three experimental sites had different types of impact on the 100 Ct/Cr index. The coefficients of variation for 100 Ct/Cr were higher in the managed grasslands than in the unmanaged grasslands. The higher seasonal variability in the 100 Ct/Cr values in the case of managed grassland is expected and desirable in an index that depends on management and not on parameters related to climate.

Additional studies must be carried out to investigate what drives seasonal changes in the 100 Ct/Cr ratio, as so far we can only dismiss the importance of soil temperature and soil moisture on the 100 Ct/Cr ratio. Thus, we hypothesize that management practices, seasonality in the production of root exudates or differences in substrate availability may be responsible for short term differences (annual variation). It is important to notice that this biochemical index appears to be rather dependent on soil management, both in the long term (comparing the managed and unmanaged grasslands) and in the short term.

In summary, the present results show that the soil biochemical properties under study responded to management and sampling date throughout the year, although the 100 Ct/Cr index was independent of annual variation. As temperature and soil humidity

were monitored at the same time, a more complete, integrated picture of the dynamics of soil biochemical properties was obtained than in previous studies.

Conclusions

Consistent differences in biochemical activities were observed in relation to different management practices. The biochemical activity was higher in unmanaged grasslands than in managed grasslands throughout the study period. The data show that soil biochemical properties exhibit a high degree of intra-annual variation, although it was not possible to identify any patterns of variation for most of the properties measured. In general, temperature, rather than soil humidity, drives the variations in some biochemical properties that are at least partly dependent on abiotic factors, although there are exceptions to this (dehydrogenase, invertase). Location of the sampling site was an important factor in determining soil biochemical properties, possibly as a result of differences in soil physical and chemical characteristics.

An index was used to evaluate biochemical equilibrium in managed and unmanaged grasslands. Although this index was also subject to variation, which appeared to be independent of soil temperature or humidity, it proved to be a powerful tool for evaluating soil biochemical equilibrium in temperate grassland ecosystems.

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Table 1. General properties of the six soils analysed (mean values and standard deviations for general soil properties determined throughout one year). MAT:mean annual temperature. TAP : total annual precipitation.

Soil	Parent material	MAT	TAP	Carbon (%)	Nitrogen (%)	pH (H_2O)	pH (KCl)	Texture
Boimorto managed	Schist	12.8 ºC	1262 mm	6.36±1.35	0.524±0.091	5.46±017	4.27±0.13	Sandy-loam
Boimorto unmanaged	Schist	12.8 ºC	1262 mm	7.32±1.29	0.526±0.118	5.08±0.25	3.93±0.11	Loam
Trabada managed	Slate	10.1 ºC	1475 mm	4.39±0.84	0.366±0.032	5.00±0.21	3.83±0.14	Loam
Trabada unmanaged	Slate	10.1 ºC	1475 mm	7.73±0.99	0.606±0.055	5.13±0.16	3.96±0.10	Loam
Rodeiro managed	Schist	9.1 ºC	1044 mm	7.75±0.81	0.669±0.042	5.28±0.21	4.14±0.11	Sandy-clay-loam
Rodeiro unmanaged	Schist	9.1 ºC	1044 mm	10.00±0.76	0.871±0.042	5.37±0.16	4.28±0.11	Sandy-clay-loam

Table 2. ANOVA results, showing the influence of soil moisture, temperature, location and management on soil biochemical properties.

Source of variation	Dependent variable	df	F	*P*
Soil moisture	Biomass-C	1	0.083	0.774
	Labile C	1	1.023	0.315
	Basal respiration	1	0.003	0.956
	Mineralised N	1	0.091	0.764
	Catalase	1	0.744	0.391
	Dehydrogenase	1	13.800	0.000
	Cellulase	1	1.230	0.271
	ß-glucosidase	1	1.858	0.177
	Invertase	1	6.604	0.012
	Casein-protease	1	0.140	0.710
	BAA-protease	1	1.333	0.252
	Urease	1	3.685	0.059
	Phosphodiesterase	1	2.475	0.120
	Phosphomonoesterase	1	0.652	0.422
	Arylsulphatase	1	2.914	0.092
Temperature	Biomass-C	1	5.947	0.017
	Labile C	1	5.655	0.020
	Basal respiration	1	81.706	0.000
	Mineralised N	1	18.627	0.000
	Catalase	1	0.087	0.769
	Dehydrogenase	1	3.867	0.053
	Cellulase	1	0.189	0.665
	ß-glucosidase	1	1.757	0.190
	Invertase	1	1.260	0.266
	Casein-protease	1	3.107	0.083
	BAA-protease	1	2.750	0.102
	Urease	1	3.837	0.054
	Phosphodiesterase	1	7.477	0.008
	Phosphomonoesterase	1	8.283	0.005
	Arylsulphatase	1	18.689	0.000
Location	Biomass-C	2	45.835	0.000
	Labile C	2	9.127	0.000
	Basal respiration	2	6.335	0.003
	Mineralised N	2	10.617	0.000
	Catalase	2	9.448	0.000
	Dehydrogenase	2	29.136	0.000
	Cellulase	2	2.459	0.093
	ß-glucosidase	2	3.694	0.030
	Invertase	2	20.732	0.000
	Casein-protease	2	11.904	0.000
	BAA-protease	2	33.654	0.000
	Urease	2	28.026	0.000
	Phosphodiesterase	2	29.871	0.000

	Phosphomonoesterase	2	98.721	0.000
	Arylsulphatase	2	84.171	0.000
Management	Biomass-C	1	149.966	0.000
	Labile C	1	113.795	0.000
	Basal respiration	1	116.757	0.000
	Mineralised N	1	0.000	0.999
	Catalase	1	108.147	0.000
	Dehydrogenase	1	115.308	0.000
	Cellulase	1	14.437	0.000
	ß-glucosidase	1	48.922	0.000
	Invertase	1	130.121	0.000
	Casein-protease	1	71.793	0.000
	BAA-protease	1	39.865	0.000
	Urease	1	45.432	0.000
	Phosphodiesterase	1	160.158	0.000
	Phosphomonoesterase	1	282.206	0.000
	Arylsulphatase	1	173.752	0.000
Location * Management	Biomass-C	2	4.307	0.017
	Labile C	2	7.982	0.001
	Basal respiration	2	0.215	0.807
	Mineralised N	2	1.607	0.208
	Catalase	2	1.261	0.290
	Dehydrogenase	2	7.321	0.001
	Cellulase	2	7.204	0.001
	ß-glucosidase	2	4.021	0.022
	Invertase	2	8.909	0.000
	Casein-protease	2	7.737	0.001
	BAA-protease	2	1.077	0.346
	Urease	2	0.496	0.611
	Phosphodiesterase	2	11.267	0.000
	Phosphomonoesterase	2	2.506	0.089
	Arylsulphatase	2	28.260	0.000

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Table 3. Results of the discriminant analysis for the six soils considered in the study.

	Root 1	Root 2
Invertase	0.535	-0.771
ß-glucosidase	-0.624	0.337
Cellulase	-0.292	0.641
Phosphomonoesterase	1.063	0.572
Phosphodiesterase	-0.343	1.268
Arylsulphatase	0.585	-1.669
Labile C	-0.012	0.701
Mineralised N	-0.419	-0.666

Table 4. ANOVA results, showing the influence of soil moisture, temperature, location and management on the Ct/Cr index

Source	df	F	P
Precipitation	1	1.209	0.276
Temperature	1	0.418	0.520
Location	2	28.611	0.000
Management	1	17.375	0.000
Location * Management	2	20.678	0.000

Figure captions:

Fig. 1 Climatograms for the three locations considered and throughout the period of study.

Fig. 2 Variation in several biochemical activities in the managed grassland (filled circles) and the unmanaged grassland (open circles) at Boimorto.

Fig. 3 Variation in several biochemical activities in the managed grassland (filled circles) and the unmanaged grassland (open circles) at Trabada.

Fig. 4 Variation in several biochemical activities in the managed grassland (filled circles) and the unmanaged grassland (open circles) at Rodeiro.

Fig. 5 Factor analysis of biochemical properties in the six soils analysed. Values indicated by the same small letter are not significantly different, according to the LSD test (P = 0.05).

Fig. 6 Discrimination of the soils analyzed according to soil biochemical variables depicted on a Root 1 x Root 2 biplot.

Fig. 7 Annual variation in the 100 Ct/Cr ratio in the six soils studied.

Fig. 1

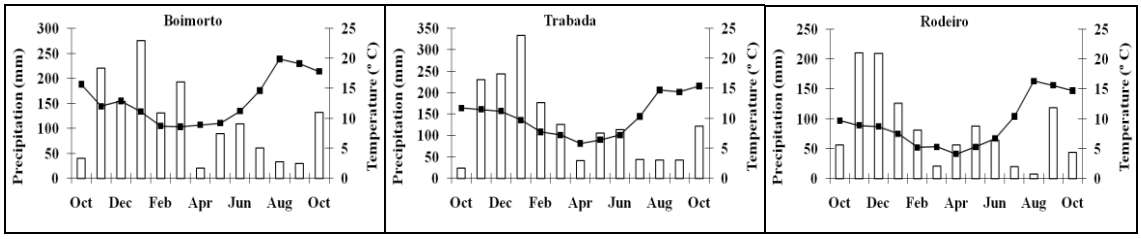

Boimorto
Trabada
Rodeiro
Precipitation (mm)
Temperature (° C)
Oct
Dec
Feb
Apr
Jun
Aug
Oct

Fig. 2

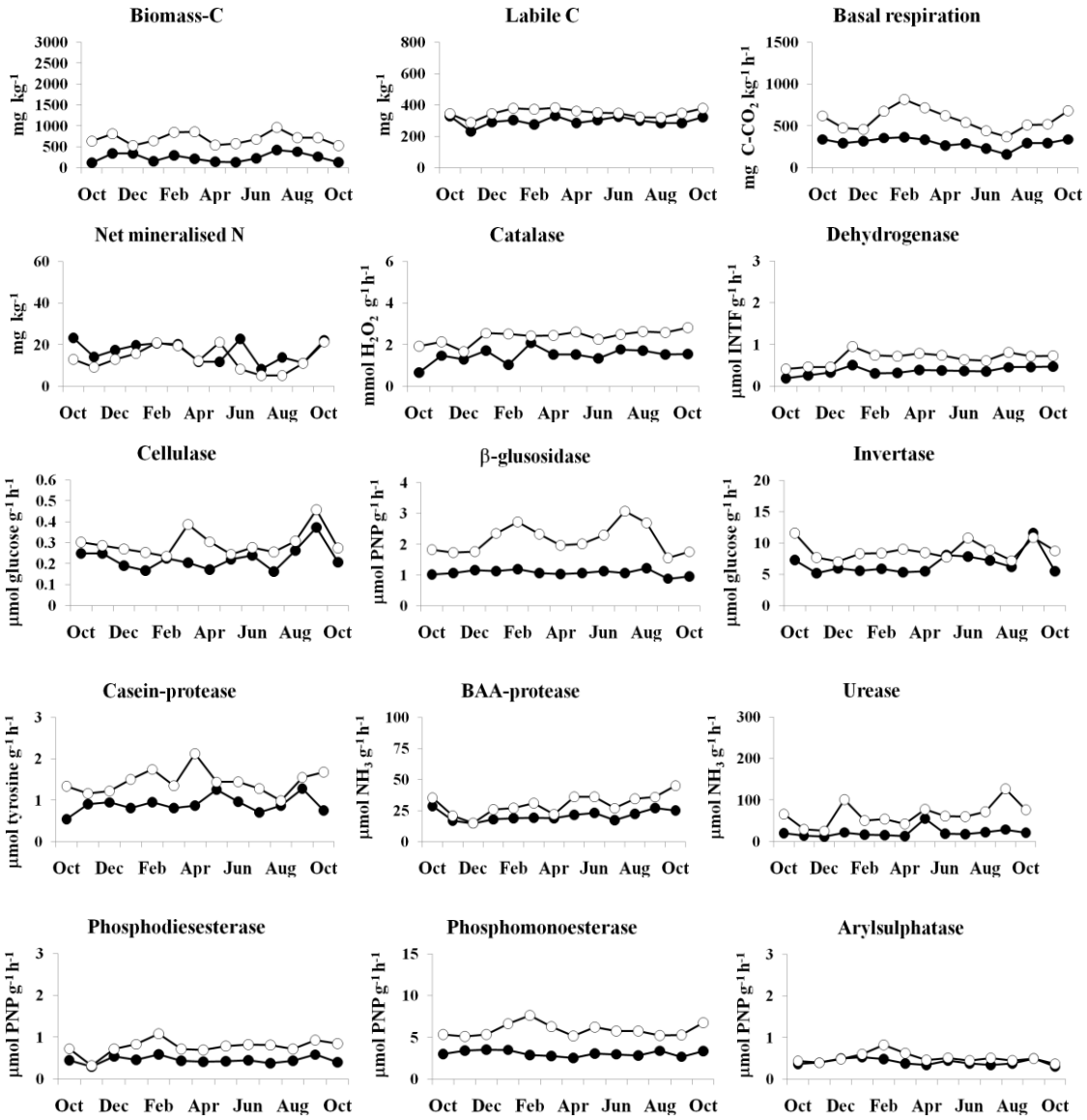

Biomass-C
mg kg-1
Labile C
mg kg-1
Basal respiration
mg C-CO2 kg-1 h-1
Net mineralised N
mg kg-1
Catalase
mmol H2O2 g-1 h-1
Dehydrogenase
µmol INTF g-1 h-1
Cellulase
µmol glucose g-1 h-1
β-glusosidase
µmol PNP g-1 h-1
Invertase
µmol glucose g-1 h-1
Casein-protease
µmol tyrosine g-1 h-1
BAA-protease
µmol NH3 g-1 h-1
Urease
µmol NH3 g-1 h-1
Phosphodiesesterase
µmol PNP g-1 h-1
Phosphomonoesterase
µmol PNP g-1 h-1
Arylsulphatase
µmol PNP g-1 h-1
Oct Dec Feb Apr Jun Aug Oct

Fig. 3

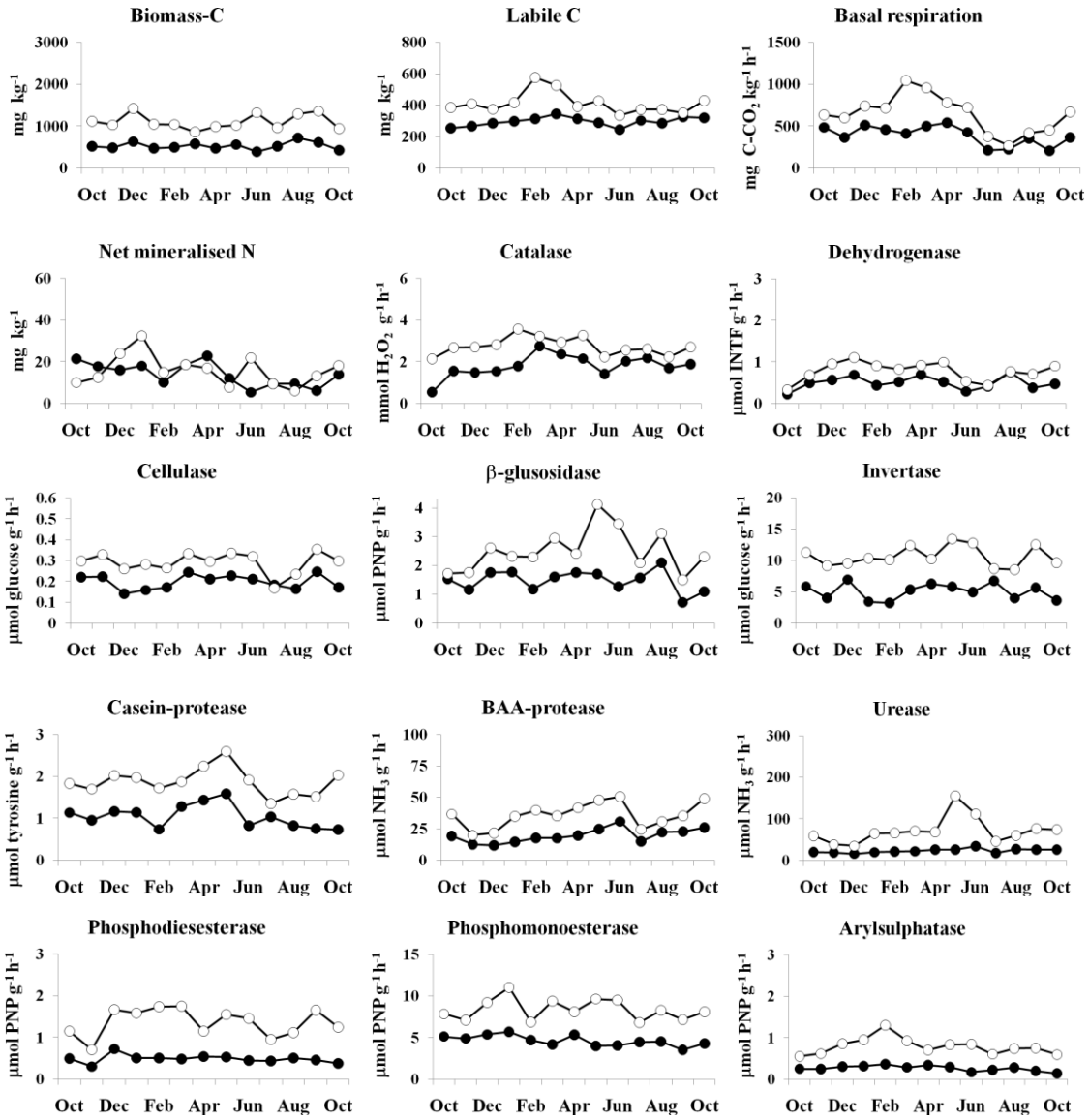

Biomass-C
mg kg-1
Labile C
mg kg-1
Basal respiration
mg C-CO2 kg-1 h-1
Net mineralised N
mg kg-1
Catalase
mmol H2O2 g-1 h-1
Dehydrogenase
µmol INTF g-1 h-1
Cellulase
µmol glucose g-1 h-1
β-glusosidase
µmol PNP g-1 h-1
Invertase
µmol glucose g-1 h-1
Casein-protease
µmol tyrosine g-1 h-1
BAA-protease
µmol NH3 g-1 h-1
Urease
µmol NH3 g-1 h-1
Phosphodiesesterase
µmol PNP g-1 h-1
Phosphomonoesterase
µmol PNP g-1 h-1
Arylsulphatase
µmol PNP g-1 h-1
Oct Dec Feb Apr Jun Aug Oct

Fig. 4

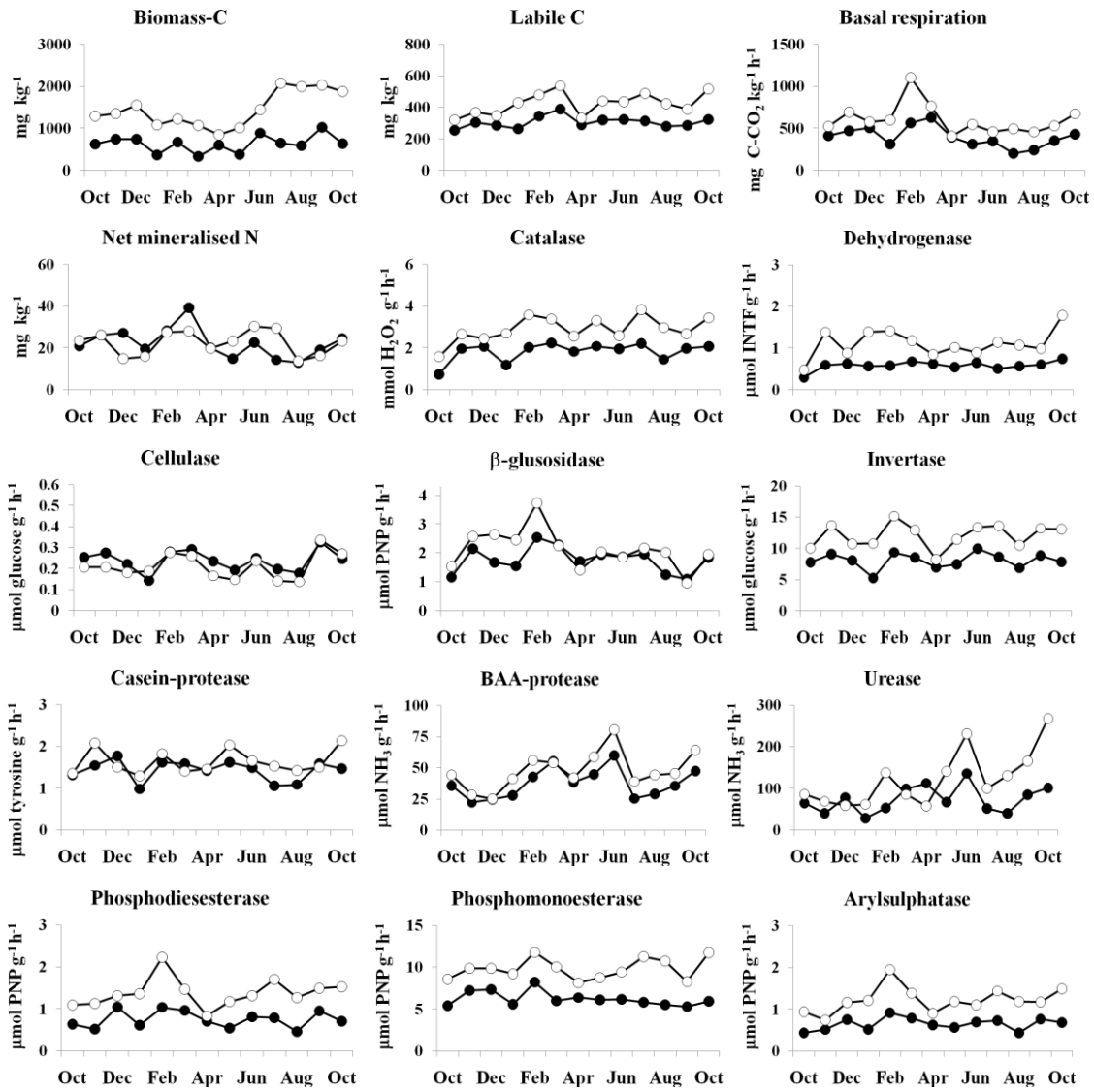
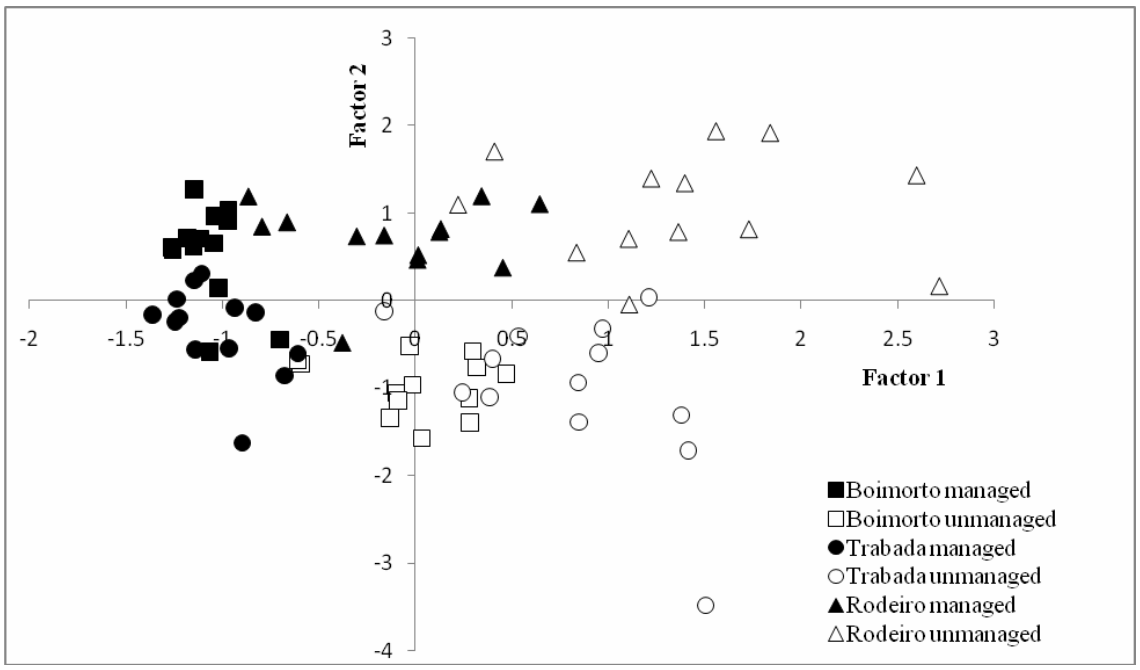
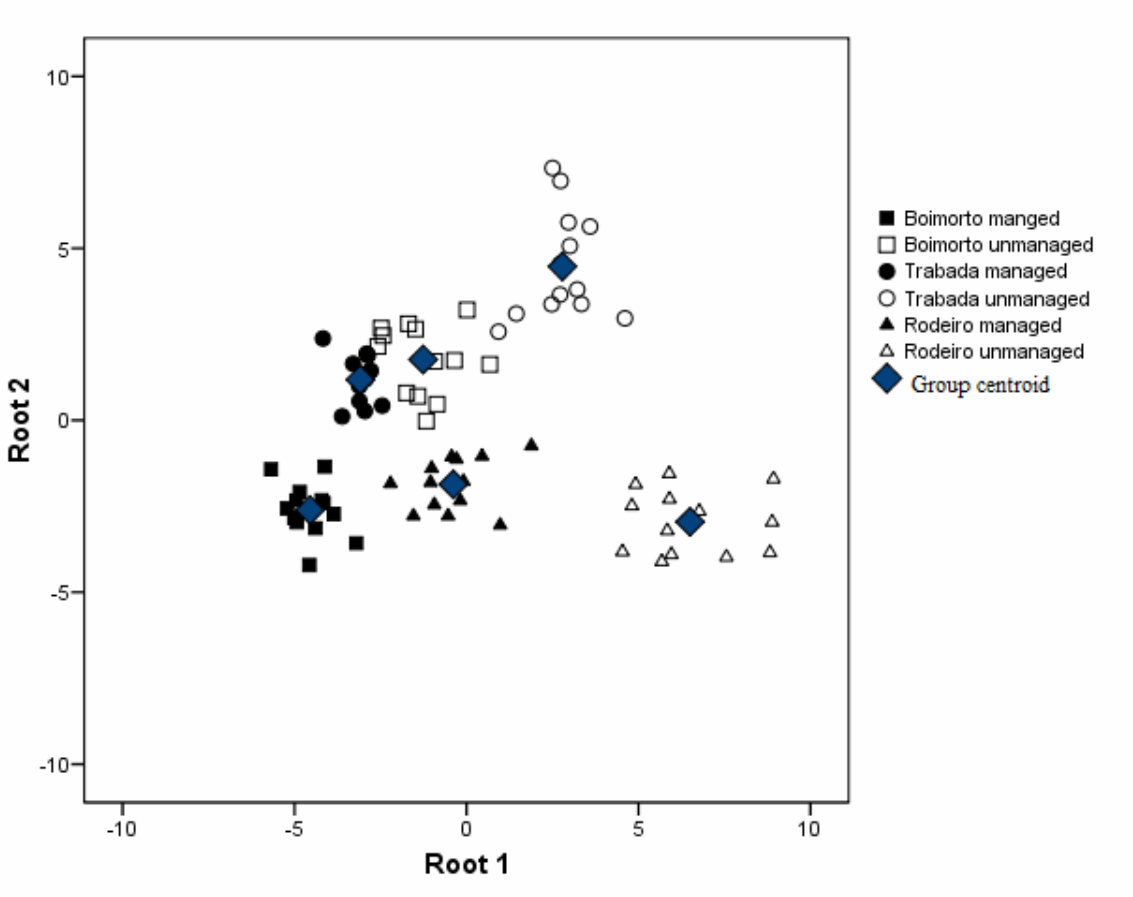

Biomass-C
mg kg-1
Labile C
mg kg-1
Basal respiration
mg C-CO2 kg-1 h-1
Net mineralised N
mg kg-1
Catalase
mmol H2O2 g-1 h-1
Dehydrogenase
µmol INTF g-1 h-1
Cellulase
µmol glucose g-1 h-1
β-glusosidase
µmol PNP g-1 h-1
Invertase
µmol glucose g-1 h-1
Casein-protease
µmol tyrosine g-1 h-1
BAA-protease
µmol NH3 g-1 h-1
Urease
µmol NH3 g-1 h-1
Phosphodiesesterase
µmol PNP g-1 h-1
Phosphomonoesterase
µmol PNP g-1 h-1
Arylsulphatase
µmol PNP g-1 h-1
Oct Dec Feb Apr Jun Aug Oct

Fig. 5



	Component		ANOVA factor 1	
	1	2	Boimorto managed	a
Organic carbon	0.822	0.253	Trabada managed	a
Organic nitrogen	0.588	0.518	Rodeiro managed	b
Biomass-C	0.812	0.120	Boimorto unmanaged	b
Labile C	0.842	-0.126	Trabada unmanaged	c
Respiration	0.746	-0.338	Rodeiro unmanaged	d
N mineralization	0.409	0.523		
Catalase	0.825	-0.279		
Dehydrogenase	0.859	0.010		
Cellulase	0.311	-0.460		
β-glucosidase	0.681	-0.436		
Invertase	0.881	-0.013		
Casein-protease	0.792	-0.348		
BAA-protease	0.745	0.191		
Urease	0.776	0.151		
Phosphodiesterase	0.925	-0.050		
Phosphomonoesterase	0.923	0.055		
Arylsulphatase	0.899	0.244		

Fig. 6


Boimorto manged
Boimorto unmanaged
Trabada managed
Trabada unmanaged
Rodeiro managed
Rodeiro unmanaged
Group centroid
Root 1
Root 2
10
5
0
-5
-10
-10
-5
0
5
10

641 Fig. 7

642

643

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